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Identification of *Candida nivariensis* and *Candida bracarensis* in a Large Global Collection of *Candida glabrata* Isolates: Comparison to the Literature▽

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We analyzed 1,598 *Candida glabrata* isolates for the presence of the cryptic species *Candida nivariensis* and *Candida bracarensis*. Both species were very rare in this collection (0.2% prevalence), despite the number of isolates analyzed and the global distribution of the isolates. We saw no associated antifungal resistance in *C. nivariensis*.

As our methods of species identification have shifted from more traditional phenotypic methodologies to nucleic acid-based techniques, previously unrecognized "cryptic" species have been added to the list of human pathogens. Recent reports have added the *Candida glabrata* phenotypic mimics *Candida nivariensis* and *Candida bracarensis* to this list (2, 5). Following a report on 16 isolates from the United Kingdom which indicated that *C. nivariensis* was an emerging pathogen with multidrug resistance (4, 6), we sought to use our unique global collection of *Candida* isolates to determine what percentage of *C. glabrata* isolates were *C. nivariensis* and *C. bracarensis* and whether these isolates indeed had high rates of resistance to antifungal drugs.

All isolates were collected as part of the ARTEMIS antifungal surveillance program between 2001 and 2006 and identified as *C. glabrata* by the Vitek yeast identification system (BioMérieux, Durham, NC). A total of 1,598 isolates phenotypically identified as *C. glabrata* were included in this study. These isolates were distributed globally. Isolates were received from 98 medical centers in 28 countries on six continents (Table 1). All isolates were plated on BBL CHROMagar, and their color was scored as either mauve or white. All isolates were then analyzed by *C. glabrata*-specific PCR with the primers CGL1 (5′-TTATCACACGACTCGACACT-3′) and CGL2 (5′-CCCACATACTGATATGGCCTACAA-3′) as described previously (1, 7).

When the 1,598 presumed *C. glabrata* isolates were plated on CHROMagar, 1,584 were mauve and 14 were white. Out of the 1,598 tested by *C. glabrata*-specific PCR, 1,595 were positive, including 11 of the 14 that were white on CHROMagar. All 14 of the isolates that were white on CHROMagar were analyzed by peptide nucleic acid fluorescence in situ hybridization (PNA-FISH) first with a probe that recognized *C. glabrata* and the two closely related species and then with probes specific for *C. nivariensis* or *C. bracarensis* (3, 9). The 11 isolates white on CHROMagar that were also *C. glabrata* PCR positive were also confirmed to be *C. glabrata* by PNA-FISH. Of the three isolates that were white on CHROMagar and negative by *C. glabrata* PCR, one was determined to be *C. nivariensis* and two were determined to be *C. bracarensis* by PNA-FISH. *C. nivariensis* and *C. bracarensis* isolates represented only 0.2% of the total *C. glabrata* complex isolates. The two *C. bracarensis* isolates originated in the United States at a single site in Wilmington, DE, but one was isolated from sputum in 2002 and the other was isolated from a bloodstream infection in 2004. The *C. nivariensis* isolate was from the pleural fluid of a patient in Westmead, Australia.

To date, the total number of published *C. bracarensis* and *C. nivariensis* isolates is small. The first three *C. nivariensis* isolates were reported from the Canary Islands in 2005 from a pulmonary abscess, blood, and urine (1). Since then, single isolates have been reported from a catheter-associated bloodstream infection in Japan and as a cause of oropharyngeal candidiasis in an AIDS patient in Indonesia (6, 12). An additional 16 isolates were reported from the United Kingdom, and 9 of these were from sterile body sites, including blood, ascitic fluid, and peritoneal fluid (4). Another isolate was recently recovered from the blood of a patient in Atlanta, GA (M. Brandt, A. Balajee, and S. Lockhart, unpublished observations).

The number of *C. bracarensis* isolates is even lower. Follow-

TABLE 1. Numbers and origins of isolates used in this study

Continent	No. of sites submitting isolates	Total no. of isolates		
		C. glabrata	*C. nivariensis*	*C. bracarensis*
North America	36	838	0	2
South America	13	133	0	0
Europe	32	400	0	0
Asia	10	111	0	0
Australia	2	42	1	0
Africa	5	71	0	0
Total	98	1,595	1	2

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TABLE 2. MICs for the non-*C. glabrata* isolates from global surveillance

Species	MIC (μg/ml)				
	Fluconazole	Amphotericin B	Caspofungin	Anidulafungin	Micafungin
C. nivariensis	2	1	0.06	0.06	0.015
C. bracarensis no. 1	16	8	0.03	0.06	0.015
C. bracarensis no. 2	2	1	0.03	0.06	0.015

ing the publication of the first two isolates as a cause of vulvovaginal candidiasis in Portugal and from a blood culture in the United Kingdom in 2006 (5), only three more have been published, isolates from the throat and stool of two oncology patients and from a pelvic abscess, all from Baltimore, MD (3). One other isolate has been recovered from a recent study of *C. glabrata* bloodstream isolates from a 1992 surveillance in San Francisco, CA (J. Frade and S. Lockhart, unpublished observations).

Antifungal susceptibility testing of the *C. nivariensis* and *C. bracarensis* isolates was performed by broth microdilution with fluconazole, caspofungin, anidulafungin, and micafungin and by Etest for amphotericin B as outlined in Clinical and Laboratory Standards Institute document M27-A2 and as previously described (8, 10) All MICs were determined visually. Echinocandin MICs were determined at 24 h, while azole and amphotericin B values were determined at 48 h. Despite reports of almost uniform azole resistance in isolates of *C. nivariensis* (4, 6), our *C. nivariensis* isolate from Australia exhibited neither azole nor echinocandin resistance (Table 2). The same was reported for the isolate from Indonesia (12) However, one of the *C. bracarensis* isolates reported here had an Etest amphotericin B MIC of 8 μg/ml (Table 2).

The study described in this report is the largest to date which has looked at the prevalence of *C. nivariensis* and *C. bracarensis* on a global scale. Despite the analysis of 1,598 *C. glabrata* isolates from 28 countries, our data indicate that *C. nivariensis* and *C. bracarensis* isolates make up only a very small percentage of the *C. glabrata* clinical isolates from global surveillance. Other published data indicate that they may be more locally or regionally prevalent (3, 4). Colony color on CHROMagar may be a very good way to rule *C. nivariensis* and *C. bracarensis* isolates in or rule out among *C. glabrata* clinical isolates. While not all of the *Candid*a isolates that were white on CHROMagar were *C. bracarensis* or *C. nivariensis*, all of the *C. nivariensis* and *C. bracarensis* isolates have been white on CHROMagar. Because of a documented increase in their incidence, a documented rise in the MICs of many antifungal drugs toward *C. glabrata* (11), and the propensity of at least some isolates of *C. nivariensis* and *C. bracarensis* to exhibit antifungal resistance, it would be prudent to continue to monitor for these emerging pathogens.

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